

**WO02056879**

Publication Title:

USE OF TERPENES FOR THE TREATMENT OF DIGESTIVE TRACT  
INFECTIONS

Abstract:

The prevention and treatment of digestive tract infections in humans and animals by orally administering a single terpene, a terpene mixture or a liposome-terpene (s) composition before or after the onset of a gastro-intestinal infection is described. Such infections may include traveller's diarrhea, ulcers, anthrax and other bacterial and parasitical infections.

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
25 July 2002 (25.07.2002)

PCT

(10) International Publication Number  
**WO 02/056879 A1**

(51) International Patent Classification<sup>7</sup>: **A61K 31/11**,  
31/405 // (A61K 31/11, 31:045)

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(21) International Application Number: PCT/GB02/00015

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(22) International Filing Date: 3 January 2002 (03.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0100059.5 3 January 2001 (03.01.2001) GB  
0108246.0 2 April 2001 (02.04.2001) GB  
60/326,818 4 October 2001 (04.10.2001) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VN, YU, ZA, ZM, ZW.

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(84) Designated States (*regional*): ARIPO patent (GI, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,  
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent  
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
NE, SN, TD, TG).

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Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

WO 02/056879 A1

(54) Title: USE OF TERPENES FOR THE TREATMENT OF DIGESTIVE TRACT INFECTIONS

(57) Abstract: The prevention and treatment of digestive tract infections in humans and animals by orally administering a single terpene, a terpene mixture or a liposome-terpene (s) composition before or after the onset of a gastro-intestinal infection is described. Such infections may include traveller's diarrhea, ulcers, anthrax and other bacterial and parasitical infections.

## USE OF TERPENES FOR THE TREATMENT OF DIGESTIVE TRACT INFECTIONS

1  
2  
3  
4 The present invention relates to the treatment of  
5 microbial infections, especially the prevention and  
6 treatment of digestive tract infections in humans  
7 and animals, by orally administering a single  
8 terpene, a terpene mixture or a liposome-terpene(s)  
9 composition before or after the onset of the  
10 infection.  
11  
12 Digestive tract infections are mainly caused by  
13 pathogenic and opportunistic microorganisms and  
14 toxins produced by them. These illnesses are  
15 present in all types of animals and humans.  
16  
17 Diseases caused by organisms pathogenic to humans  
18 and animals are very common and encompass a range  
19 from the trivial to the lethal. With the arrival  
20 of the so-called 'antibiotic age' following World  
21 War II, it was hoped that the scourge of infection

1 would be I largely controlled on a permanent basis.  
2 However, this has not proved to be the case and in  
3 recent years many formerly useful prior art  
4 anti-bacterials have become ineffective as  
5 resistance has emerged. In the case of fungal  
6 infections the armamentarium has always been  
7 limited and the need remains for additional and  
8 more effective treatments.

9  
10 In recent years, a number of particularly difficult  
11 problems have emerged and these have engaged  
12 considerable public concern. For instance, the  
13 rapidly rising prevalence of multiply resistant  
14 *Staphylococcus aureus* (MRSA) in hospitals in  
15 Western countries which has led to many deaths and,  
16 to all intents and purposes, only Vancomycin now  
17 stands as a fall-back treatment. Another example  
18 is outbreaks of severe *E. coli* infection, such as  
19 that in Scotland in the late nineteen-nineties  
20 which killed over 150 people. In the case of *E.*  
21 *coli*, there are particular problems in respect of  
22 treatment in that, even if the organism is killed  
23 quickly, the patient may die as the result of  
24 endotoxins being released from the organism if it  
25 is lysed as a result of anti-microbial attack.

26  
27 Not all the mechanisms governing the emergence of  
28 resistance to anti-bacterials are understood but  
29 sufficient is known to suggest strongly that whilst  
30 a fairly simple game of molecular roulette will  
31 produce new anti-bacterials, any such product will

1 not remain free of resistance for long. Thus, it  
2 would appear that any solution to this apparently  
3 intractable problem of reduced effectiveness in  
4 anti-bacterials would need to be radically  
5 different to those employed in the prior art.

6  
7 Recently with the scare of bio-terrorism there has  
8 been an increased concern with pathogens that can  
9 produce deadly outbreaks. This is the case with  
10 anthrax. Anthrax is considered a potential agent  
11 for use in biological warfare. Anthrax is an acute  
12 infectious disease caused by the spore-forming  
13 bacteria *Bacillus anthracis*. Anthrax is primarily  
14 a disease of domesticated and wild animals,  
15 particularly herbivorous animals. Humans become  
16 infected with anthrax by handling products from  
17 infected animals or by inhaling anthrax spores from  
18 contaminated animal products. Anthrax can also be  
19 spread by eating undercooked meat from infected  
20 animals. Anthrax infection can occur in three  
21 forms: cutaneous, inhalation, and gastrointestinal.  
22 The most common form is the cutaneous anthrax  
23 infection, which occurs when bacteria enter a cut  
24 or abrasion on the skin. This infection begins as  
25 a raised itchy bump that develops into a vesicle  
26 and then a painless ulcer, usually 1-3 cm in  
27 diameter, with a characteristic black necrotic area  
28 in the center. About 20% of untreated cases of  
29 cutaneous anthrax result in death. Deaths may be  
30 prevented with prompt antimicrobial treatment. The  
31 inhalation form has early symptom similar to a

1 common cold which progressively results in severe  
2 breathing problems. This type of anthrax is  
3 usually fatal. The intestinal form is  
4 characterized by an acute inflammation of the  
5 intestinal tract. The initial signs are nausea,  
6 loss of appetite, vomiting, and fever followed by  
7 abdominal pain, vomiting of blood and severe  
8 diarrhea. Intestinal anthrax results in death in  
9 25% to 60% of cases. Anthrax is treated with  
10 antimicrobials and can be prevented with  
11 vaccination. The Department of Defense in the USA  
12 has a mandatory anthrax vaccination of all active  
13 military personnel.

14  
15 Another digestive infection in humans is  
16 traveller's diarrhea, which affects over seven  
17 million visitors to high-risk tropical and  
18 semitropical areas every year. Others suggest that  
19 the incidence of traveller's diarrhea is 15 - 56%  
20 among international travelers. Approximately 1% of  
21 the sufferers are hospitalized, at least 20% are  
22 confined to bed for a day and nearly 40% have to  
23 change plans in their travel itinerary.

24  
25 Traveler's diarrhea, defined as the passage of more  
26 than 3 unformed stools in a 24-hour period, is a  
27 self-limiting illness lasting 3 - 5 days. The  
28 illness may be presented either as (1) acute watery  
29 diarrhea (2) diarrhea with blood (dysentery) or (3)  
30 chronic diarrhea, often with clinical nutrient  
31 malabsorption.

1 Several factors contribute to the development of  
2 diarrhea in travelers, including personal (age,  
3 socioeconomic status, body weight, preexisting  
4 gastrointestinal illnesses), behavioral (mode of  
5 travel, standard of accommodation, eating in public  
6 places, dietary errors) and travel related  
7 (destination, duration of stay, country of origin,  
8 season). Approximately 85% of the diarrheas  
9 among international travelers are produced by  
10 bacterial enteropathogens. These pathogens are  
11 usually acquired through ingestion of fecally  
12 contaminated food or water. Sometimes dirty hands  
13 or insects are the vectors of fecal contamination.  
14 Cooked food is safe to consume as long as the  
15 temperature at the interior of the food reaches  
16 160°F or more. An undercooked hamburger is risky  
17 food, because ground meat can become contaminated  
18 at the processing plant and during preparation.

19

20 The common pathogens that produce traveler's  
21 diarrhea include *Clostridium difficile*, *Yersenia*  
22 *enterocolitica*, *Shigella* sp., *Campylobacter* sp.,  
23 *Salmonella* sp., ETEC (enterotoxigenic) and EAEC  
24 (enteroaggregative) *Escherichia coli*. Traveler's  
25 diarrhea produced by *Shigella* sp. or *Salmonella* sp.  
26 tend to cause a more severe and longer lasting  
27 disease than that caused by the most common cause,  
28 enterotoxigenic *E. coli* (ETEC). *Campylobacter*  
29 *jejuni* is a relatively common cause of traveler's  
30 diarrhea especially in the winter. Viruses such as

1 rotavirus, cytomegalovirus and Norwalk agent are  
2 less common causes.

3

4 There are several groups of pathogenic *E. coli*.  
5 They include Enterotoxigenic (ETEC), which produce  
6 a range of toxins, heat-stable or heat-labile in  
7 nature. ETEC is the most common cause of diarrhoea  
8 disease in children in the developing world; it  
9 also causes many travelers' diarrhoea cases.

10

11 Verocytotoxic *E. coli* (VTEC) strains produce toxins  
12 that destroy the gut mucosa and can cause kidney  
13 damage; *E. coli* 0157 H:7 is the most publicised  
14 example of this type.

15

16 Enteropathogenic *E. coli* (EPEC) do not appear to  
17 produce toxins but may attach the microvilli, this  
18 group often causes infection in babies and young  
19 children.

20

21 Enteroinvasive *E. coli* (EIEC) attaches to the  
22 mucosal lining of the large intestine and invade  
23 the cells, causing tissue destruction and  
24 inflammation. EIEC are usually food borne  
25 pathogens and are an important cause of disease in  
26 areas of poor hygiene.

27

28 The severity of the disease symptoms are dependent  
29 on the strain encountered and the underlying health  
30 of the individual. EIEC and VTEC strains can cause  
31 very serious disease (haemorrhagic colitis and

1 renal failure) and require hospitalisation. Milder  
2 cases are usually treated by fluid and electrolyte  
3 replacement and rest.

4

5 The use of antibiotics limits the course of  
6 diarrhea to a little over a day compared with an  
7 average of over 3 - 5 days when diarrhea remains  
8 untreated. The widespread resistance of the  
9 traditional antimicrobial agent, Trimethoprim plus  
10 sulfamethoxazole (TMP/SMX), and fluoroquinolones  
11 are the main reasons of concern about the  
12 continuous use of antimicrobials for the treatment  
13 of traveler's diarrhea (Dupont et al, 1998). The  
14 extensive use of antibiotics can also lead to  
15 overgrowth syndromes, *Candida vaginitis* can occur,  
16 the overgrowth of *Clostridium difficile* due to less  
17 competitive environment in the gastrointestinal  
18 tract can also result in diarrhea.

19

20 Short-term travelers that have experience diarrhea  
21 do not develop protection, since it requires  
22 continued exposure to enteropathogens to develop  
23 immunological protection against traveler's  
24 diarrhea. Vaccination is a promising option, but  
25 vaccines against all enteropathogens that cause  
26 traveler's diarrhea have not been developed. Other  
27 protection methods to treat traveler's diarrhea  
28 are: the use of nonabsorbed antimicrobials, which  
29 have fewer side effects and should be safer to use  
30 in children and pregnant women in whom quinolones  
31 are contraindicated; antisecretory and antimotility

1 agent (loperamide); the use of attapulgite, a  
2 hydrated aluminum silicate clay preparation; and  
3 probiotics i.e. lactobacillus, which appear to be  
4 useful in the prevention or treatment of travelers  
5 diarrhea. In all cases the restoration of water  
6 and electrolyte balance is necessary. The following  
7 table shows the current treatments for Traveler's  
8 Diarrhea:

Agent	Efficacy	Comments
Activated charcoal	Not efficacious	May absorb important medications
Lactobacillus	Not proven	Safe
Bismuth subsalicylate preparations	65% protective	Rinse mouth to avoid black tongue
Trimethopim- Sulfamethoxazole	70-80% protective	Resistance rising worldwide
Fluoroquinolones (norfloxacin, ciprofloxacin, Ofloxacin)	90% protective or better	Currently most effective antimicrobial but resistance rising worldwide.

10 Ericsson, Charles (1998)

11

12 In humans and animals, peptic ulcers are open sores  
13 produced by a bacteria. These open sores can be  
14 present on the entire gastro-intestinal tract,  
15 mainly esophagus, stomach and proximal part of the  
16 small intestine. There is evidence that support  
17 the role of *H. pylori* as the etiologic agent of  
18 chronic gastritis and peptic ulcer. *H. pylori*, a

1 gram-negative, microaerophilic spiral bacteria is  
2 the major cause of gastro-duodenal disease,  
3 including chronic gastritis, gastric and duodenal  
4 ulcers and gastric neoplasia. Greater than 50% of  
5 North American adults over 50 years of age are  
6 infected with *H. pylori*. In contrast, in some  
7 developing and newly industrialized countries  
8 virtually all adults are infected. In developing  
9 countries almost all children are infected by age  
10 10, whereas in developed countries only the  
11 children of lower socioeconomic levels are  
12 infected. *H. pylori* is characterized by very high  
13 urease activity that may be associated with  
14 virulence, in the absence of urea *H. pylori* is  
15 sensitive to acidic pH. Urease activity may be an  
16 important colonization and survival factor by  
17 generating ammonia in the immediate bacterial  
18 microenvironment. *H. pylori* has been classified as  
19 a type 1 carcinogen by the World Health  
20 Organization because of the danger of persistent  
21 infection with the bacterium causing gastric  
22 cancer. *H. pylori* infection is of extreme  
23 importance in the causation of peptic ulcer  
24 disease. By initiating a gastritis or dyspeptic  
25 symptoms, it can predispose to subsequent episode  
26 of either gastric lymphoma or stomach cancer.  
27  
28 The eradication of *H. pylori* has been obtained with  
29 combination therapy, triple therapy using bismuth  
30 plus two antibiotics (metronidazole and either  
31 amoxicillin or tetracycline has been effective).

1 Problems due to development of antimicrobial  
2 resistant and side effects (diarrhea, nausea,  
3 abdominal pain and others) may explain why the use  
4 of antibiotics has not become a preferred treatment  
5 for gastritis and peptic ulcers due to *H. pylori*.

6  
7 Antibacterial treatment of *H. pylori* is difficult  
8 because of the habitat occupied by the organism  
9 below the layer of the mucus adherent to the  
10 gastric mucosa. Access of antibacterial agents to  
11 this site is limited from the lumen of the stomach  
12 and also from the gastric blood supply.

13  
14 The use of medium chain fatty acids and medium  
15 chain triglycerides has been shown to inhibit the  
16 growth of *H. pylori* in vitro. The mechanism by  
17 which they exert antibacterial effect is thought to  
18 involve: 1) damage to the bacterial outer membrane  
19 leading the increase membrane fluidity and  
20 permeability, 2) Incorporation of these fatty  
21 acids, making the bacterial membrane unstable, 3)  
22 Production of peroxides due to oxidation of fatty  
23 acids.

24  
25 The mode of transmission of *H. pylori* in humans is  
26 still poorly understood. There are reports of  
27 detection of this microorganism in the oral cavity  
28 and in the feces. If *H. pylori* is harvested in the  
29 oral cavity or bowel, these might represent  
30 important reservoir for the reinfection and  
31 transmission with consequences from treatment. One

1 vector for the transmission of *H. pylori* are flies,  
2 they can carry viable *H. pylori* in their external  
3 surfaces and alimentary tracts.

4

5 In animals, the presence of scours in calves is of  
6 economic importance. It is estimated that the  
7 death lost of calves less than 6 months of age is  
8 approximately 2.5% or over 100,000 a year. Most of  
9 the mortality and morbidity of the calves are due  
10 to infectious diseases, mainly scours. More than  
11 90% of scours in calves is produced by *E. coli* and  
12 *Salmonella*. Clostridia has proved to be fatal in  
13 the majority of cases. There are preventive  
14 methods like (1) vaccination of the mothers in  
15 order to passively transfer antibodies in  
16 colostrum; (2) the use of immunological supplements  
17 for milk replacers; (3) the use of probiotics to  
18 create a gastro-intestinal healthy environment (4)  
19 changes in calf management. None of these  
20 protective measures are 100% effective.

21

22 Another animal of economic importance is swine.  
23 The incidence of diarrhea in neonates and weaned  
24 piglets is very high. Again, *E. coli* and  
25 *Salmonella* are the main microorganisms involved in  
26 diarrhea in swine. There are losses in the nursery  
27 while piglets are still lactating and after  
28 weaning. There are similar preventive methods as  
29 in calves. One of the preferred methods is  
30 segregated early weaning (SEW). The basis of early  
31 weaning is that the earlier piglets are weaned from

1 the sow the less are the chances of crossover  
2 diseases between sow and piglets. This method  
3 requires the use of antibiotics.

4

5 In both cases, calf and piglet scours, the  
6 preferred method of treatment is antibiotics. The  
7 European Community has banned the use of 5  
8 antibiotics and in the United States the FDA is  
9 banning the use of fluoroquinolone in animals due  
10 to the development of *Campylobacter* resistant to  
11 this antibiotic. Bacteria resistance has  
12 encouraged the development of antibiotic-  
13 alternative products.

14

15 Terpenes are widespread in nature, mainly in plants  
16 as constituents of essential oils. Their building  
17 block is the hydrocarbon isoprene  $(C_5H_8)_n$ . Terpenes  
18 have been found to be effective and nontoxic  
19 dietary antitumor agents which act through a  
20 variety of mechanisms of action (Crowell and Gould,  
21 1994 and Crowell et al, 1996). Terpenes, i.e.  
22 geraniol, tocotrienol, perillyl alcohol,  $\beta$ -ionone  
23 and d-limonene, suppress hepatic HMG-CoA reductase  
24 activity, a rate limiting step in cholesterol  
25 synthesis, and modestly lower cholesterol levels in  
26 animals (Elson and Yu, 1994). D-limonene and  
27 geraniol reduced mammary tumors (Elegbede et al,  
28 1984 and 1986 and Karlson et al, 1996) and  
29 suppressed the growth of transplanted tumors (Yu et  
30 al, 1995).

31

1 Terpenes have also been found to inhibit the  
2 in-vitro growth of bacteria and fungi (Chaumont and  
3 Leger, 1992, Moleyar and Narasimham, 1992 and  
4 Pattnaik, et al, 1997) and some internal and  
5 external parasites (Hooser, et al, 1986). Geraniol  
6 was found to inhibit growth of *Candida albicans* and  
7 *Saccharomyces cerevisiae* strains by enhancing the  
8 rate of potassium leakage and disrupting membrane  
9 fluidity (Bard, et al, 1988). B-ionone has  
10 antifungal activity which was determined by  
11 inhibition of spore germination, and growth  
12 inhibition in agar (Mikhlin et al, 1983 and Salt et  
13 al, 1986). Terpenone (geranylgeranylacetone) has  
14 an antibacterial effect on *H. pylori* (Ishii, 1993).  
15 Solutions of 11 different terpenes were effective  
16 in inhibiting the growth of pathogenic bacteria in  
17 in-vitro tests; levels ranging between 100 ppm and  
18 1000 ppm were effective. The terpenes were diluted  
19 in water with 1% polysorbate 20 (Kim et al, 1995).  
20 Diterpenes, i.e. trichorabdol A (from R.  
21 *Trichocarpa*) has shown a very strong antibacterial  
22 effect against *H. pylori* (Kadota, et al, 1997).  
23  
24 Rosanol a commercial product with 1% rose oil has  
25 been shown to inhibit the growth of several  
26 bacteria (*Pseudomonas*, *Staphylococcus*, *E. coli* and  
27 *H. pylori*). Geraniol is the active component (75%)  
28 of rose oil. Rose oil and geraniol at a  
29 concentration of 2 mg/litre inhibited the growth of  
30 *H. pylori* in vitro. Some extracts from herbal  
31 medicines have been shown to have an inhibitory

1 effect on *H. pylori*, the most effective being  
2 decursinol angelate, decursin, magnolol, berberine,  
3 cinnamic acid, decursinol and gallic acid (Bae, et  
4 al 1998). Extracts from cashew apple, anacardic  
5 acid and (E)-2-hexenal, have shown bactericidal  
6 effect against *H. pylori*.

7 There may be different modes of action of terpenes  
8 against *H. pylori*. They could (1) interfere with  
9 the phospholipid bilayer of the cell membrane (2)  
10 impair a variety of enzyme systems (HMG-reductase)  
11 and (3) destroy or inactivate genetic material.

12

#### 13 SUMMARY OF THE INVENTION

14

15 Prevention and treatment of digestive tract  
16 infections by orally administering a biocidal  
17 terpene, a biocidal terpene mixture or a  
18 liposome-terpene(s) composition before of after the  
19 onset of the infection.

20

#### 21 DESCRIPTION OF THE PREFERRED EMBODIMENTS

22

23 Digestive tract infections not only are an  
24 uncomfortable illness for humans but also are of  
25 economic importance for the animal industry. In  
26 some cases the illness can cause death in children,  
27 elderly and immune-compromised people. The  
28 preferred treatment of the disease is antibiotics.  
29 The extensive use of antibiotics in humans and the  
30 animal industry has created the development of  
31 antibiotic-resistant bacteria. The increased

1 antibiotic resistance has been the main reason to  
2 seek new antimicrobial alternatives. The European  
3 Community has banned the use of 5 antibiotics in  
4 animals and in the United States the FDA is banning  
5 the use of fluoroquinolone in animals due to the  
6 development of *Campylobacter* resistant to this  
7 antibiotic.

8 Terpenes, which are GRAS (Generally Recognized As  
9 Safe) have been found to inhibit the growth of  
10 cancerous cells, decrease tumor size, decrease  
11 cholesterol levels and have a biocidal effect on  
12 microorganisms in vitro. Onawunmi (1989) showed  
13 that growth media with more than 0.01 % citral  
14 reduced the concentration of *E. coli* and at 0.08%  
15 there was a bactericidal effect. Barranx, et al  
16 (1998) teach us a terpene formulation, based on  
17 pine oil, used as a disinfectant or antiseptic  
18 cleaner. Koga, et al (1998) teach that a terpene  
19 found in rice has antifungal activity. Iyer, et al  
20 (1999) teach us an oral hygiene antimicrobial  
21 product with a combination of 2 or 3 terpenes that  
22 showed a synergistic effect. Neither of them  
23 suggested the use of a terpene, terpene mixture or  
24 liposome-terpene(s) combination for the prevention  
25 or treatment of gastro-intestinal infections i.e.  
26 traveler's diarrhea.

27

28 Several US Patents (US#5,547,677, US#5,549,901,  
29 US#5,618,840, US#5,629,021, US#5,662,957,  
30 US#5,700,679, US#5,730,989) teach us that certain

1 types of oil-in-water emulsions have antimicrobial,  
2 adjuvant and delivery properties.

3

4 Thus, the present invention provides a composition  
5 for preventing or treating gastro-intestinal  
6 infections, wherein said composition comprises a  
7 terpene or a mixture of terpenes. We have found  
8 that certain mixture of terpenes are  
9 synergistically effective, relative to the effects  
10 of the component terpenes administered separately.  
11 Thus terpenes having biocidal activity which in  
12 combination with two or more other terpenes  
13 synergistically increase the biocidal effectiveness  
14 are of especial interest.

15

16 One composition of interest comprises a mixture of  
17 carvone and geraniol, optionally together with  
18 other terpenes. The content of carvone and  
19 geraniol may each be from 10 to 90% (by weight),  
20 but is preferably 10 to 60% by weight. Other  
21 terpenes which may be present include citral, b-  
22 ionone, eugenol, terpeniol, carvacrol, anethole or  
23 the like. These optional additional terpenes may  
24 be present at 5 to 50% by weight, for example 10 to  
25 40% by weight.

26

27 Optionally, the terpenes may be presented in the  
28 form of liposomes.

29

30 Liposomes are microscopic structures consisting of  
31 concentric lipid bilayers enclosing an aqueous

1 space. Liposomes are classically prepared from  
2 phospholipids which occur naturally in animal cell  
3 membranes, but several synthetic formulations are  
4 now commonly used. The lipid composition of the  
5 liposome can be varied to give liposomes different  
6 physical characteristics i.e. size and stability.  
7 Liposomes can be prepared by the reverse-phase  
8 evaporation or dehydration-rehydration vesicle  
9 methods using a mixture of dipalmitoyl phosphatidyl  
10 choline, cholesterol, dipalmitoyl phosphatidyl  
11 glycerol, dipalmitoyl phosphatidyl ethanolamine and  
12 other synthetic fatty acids and emulsifiers. When  
13 making liposomes first multilamellar vesicles are  
14 formed spontaneously when amphipathic lipids are  
15 hydrated in an aqueous medium. Unilamellar vesicles  
16 are often produced from multilamellar vesicles by  
17 the application of ultrasonic waves.

18

19 Multilamellar vesicles can be prepared by the  
20 procedure known as dehydration-rehydration.  
21 Briefly, egg phosphatidylcholine and cholesterol  
22 are mixed in chloroform, dried in a rotary  
23 evaporator, dilute with water and sonificated to  
24 form unilamellar vesicles. The solution is freeze  
25 dried and rehydrated with the terpene solution in  
26 order to embed the terpene inside the liposome.  
27 Another method to produce liposomes is by mixing  
28 together lipids, an emulsifier and the terpenes.  
29 The emulsion is obtained by using a Polytron  
30 homogenizer with special flat rotor that creates an  
31 emulsion. The lipids could consist of soybean oil,

1 any commercial or pharmaceutical oil; the  
2 emulsifier consist of egg yolk lecithin, plant  
3 sterols or synthetic including polysorbate-80,  
4 polysorbate-20, polysorbate-40, polysorbate-60,  
5 polyglyceryl esters, polyglyceryl monooleate,  
6 decaglyceryl monocaprylate, propylene glycol  
7 dicaprilate and triglycerol monostearate. The  
8 lipid concentration in the oil phase is 75-95% and  
9 the emulsifier concentration from 5-25%. When  
10 preparing the emulsion a ratio oil to water could  
11 vary from 10-15 parts lipid to 35-40 parts terpenes  
12 diluted in water at a concentration of 0.5% to 50%.  
13 Once the emulsion is formed this is combined with a  
14 carrier in order to be use as a humectant, cream or  
15 other suitable carrier for topical applications.  
16 The emulsion concentration use for topical  
17 application varies from 0.0055 through 1.0% of the  
18 final product. Several modifications to the  
19 emulsion can be achieved by simply varying the  
20 concentration and type of terpenes used. This  
21 modification can give us different products with  
22 different antimicrobial specificity.  
23  
24 By encapsulating terpenes within these emulsions  
25 the antimicrobial effect will be increased: (1) the  
26 liposome will disrupt the bacterial membrane and  
27 (2) the terpenes will be more effective in  
28 disrupting cytoplasmatic enzymes.  
29  
30 It will be apparent for those skilled in the art  
31 that the aforementioned objects and other

1 advantages may be further achieved by the practice  
2 of the present invention.

3

4 EXAMPLE 1: Preparation of the terpene mixture

5

6 The terpene, terpene mixture or liposome-terpene(s)  
7 combination consists of a blend of generally  
8 recognized as safe (GRAS) terpenes with a GRAS  
9 surfactant. The ratio of terpenes is from 1-99%  
10 and the surfactant ratio from 1-99% of the mixture.  
11 The terpenes, comprised of natural or synthetic  
12 terpenes, are citral, b-ionone, geraniol, eugenol,  
13 carvone, terpeniol, carvacrol, anethole or other  
14 terpenes with similar properties. The surfactant is  
15 preferably polysorbate-80 or other suitable GRAS  
16 surfactants.

17

18 EXAMPLE 2: Preparation of liposomes containing  
19 terpenes

20

21 Any standard method for the preparation of  
22 liposomes can be followed with the knowledge that  
23 the lipids used are all food-grade or  
24 pharmaceutical-grade. A set amount of lipids, an  
25 emulsifier and the terpenes was used to prepare an  
26 emulsion. The emulsion was obtained by using a  
27 Polytron homogenizer with special flat rotor that  
28 created an emulsion. The lipids consisted of  
29 soybean oil, any commercial or pharmaceutical oil;  
30 the emulsifier consist of egg yolk lecithin, plant  
31 sterols or synthetic emulsifiers including

1 polysorbate-80, polysorbate-20, polysorbate-40,  
2 polysorbate-60, polyglyceryl esters, polyglyceryl  
3 monooleate, decaglyceryl monocaprylate, propylene  
4 glycol dicaprylate and triglycerol monostearate. A  
5 solution containing 75-95% lipids (oil) and 5-25%  
6 emulsifier consisted of the oil phase. The aqueous  
7 phase consisted of the terpene diluted in water at  
8 a rate of 0.5% to 50%. To form the emulsion a  
9 ratio of oil to water varying from 10-15 parts  
10 lipid (oil phase) to 35-40 parts terpenes (aqueous  
11 phase) was mixed. Any standard method for the  
12 preparation of liposomes can be followed with the  
13 knowledge that the lipids used are all food-grade  
14 or pharmaceutical-grade. The suspension containing  
15 a lipid, an emulsifier and the terpenes is  
16 emulsified with a Posytron homogenizer until a  
17 complete milky solution is obtained.

18

19 EXAMPLE 3: Preparation of liposomes

20

21 This step consists of the preparation of the  
22 terpene(s)-liposome combination by mixing 99% of  
23 liposome and 1% of terpene mixture. Several  
24 combinations of this formulation can be obtained by  
25 varying the amount of terpene and liposome from 1%  
26 to 99%. The liposomes are prepared as in Example 2  
27 without the addition of terpenes in the  
28 formulation.

29

30 EXAMPLE 4: In-vitro effectiveness of terpenes  
31 against E. coli

1 This example demonstrates the effect of terpenes on  
2 the cell membrane fragility of *E. coli*, which is  
3 considered indicative of other pathogenic bacteria  
4 such as *Salmonella* and *Listeria*. Lysis of the cell  
5 membrane was monitored by the determination of  
6 galactosidase activity.  $\beta$ -galactosidase is a  
7 well-characterized cytosolic enzyme in bacteria.  
8 This enzyme is inducible in the presence of  
9 isopropyl- $\beta$ -thiogalactoside (IPTG) and assayed  
10 colorimetrically with substrate  
11 o-nitro-phenyl- $\beta$ -D-galactoside (ONPG). ONPG is  
12 cleaved to release o-nitrophenol with peak  
13 absorbance at 420 nm. Since intact *E. coli* is  
14 impermeable to both ONPG and the enzyme, the cells  
15 have to be lysed prior to enzymatic assay.  
16 Therefore the ability of terpenes to lyse *E. coli*  
17 can be measured with this enzymatic assay and  
18 compared to known lysing agents.

19  
20 The procedure used was as follows: *E. coli* strains  
21 AW574 or AW405 were cultured overnight in 10 ml  
22 tryptone broth with 1 mM IPTG at 35°C. Cells were  
23 allowed to grow until an absorbance equal to 0.9  
24 was reached. Cells were harvested, washed with  
25 phosphate buffer and resuspended to an absorbance  
26 equal to 0.5. 0.1 ml of the bacteria culture was  
27 added to 0.9 ml of buffer, warmed to 30°C and then  
28 80  $\mu$ l of terpenes (85% terpenes and 15%  
29 polysorbate-80), 80  $\mu$ l water (background) or 40  $\mu$ l  
30 chloroform plus 40  $\mu$ l 1% SDS in water (positive

1 control) were added. After the addition of the  
2 lysing agents the tubes were mixed for 10 seconds  
3 and 0.2 ml of ONPG (4 mg/ml water) was added, then  
4 incubated for 5 minutes. The enzyme activity was  
5 stopped with 0.5 ml of 1 M sodium carbonate. After  
6 being centrifuged for 3 minutes at 1,500 x g,  
7 supernatant was transferred to cuvettes and read at  
8 420 nm. The relative degree of lysis caused by  
9 terpenes was calculated as follows:

10

11  $100 \times (\text{OD terpenes} - \text{OD water}) / (\text{OD chloroform} - \text{OD}$   
12  $\text{water})$

13

14 This shows that dosages can be manipulated to  
15 either lyse the cell outright, or in the case of  
16 lower dosages, stop bacterial growth without lysis  
17 of the cell membrane. The advantage of this  
18 controllable result is the ability to prevent lysis  
19 and the resultant release of endotoxins where  
20 contraindicated.

21

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31

1 Table 1: Lysis of *E. coli* by Terpenes

Terpenes ( $\mu\text{M}$ )		Relative lysis %
Carvone	404,000	NM*
	40,400	54
	4,040	22
	404	3.2
Geraniol	363,000	NM
	36,300	96
	3,630	98
	363	34
	36.3	4
	3.63	2.4
b-Ionone	308,000	NM
	30,800	NM
	3,080	NM
	308	52
	30.8	44
	3.08	23
	0.308	4.78
	0.0308	1.3
80 $\mu\text{l}$ Polysorbate-80		3.2
80 $\mu\text{l}$ Polysorbate-80 + SDS + Chloroform		100
SDS + Chloroform		100*

2

3 \*Lysis due to chloroform and SDS combination was  
4 considered to be 100%.

5 \*NM, not measurable due to formation of turbid  
6 colloidal solution.

1   EXAMPLE 5: In in-vitro effectiveness of terpenes  
2   against several microorganisms

3  
4   This example demonstrate the effectiveness of  
5   terpenes against *Escherichia coli*,  
6   *Salmonella typhimurium*, *Pasteurella mirabilis*,  
7   *Staphylococcus aureus*, *Candida albicans* and  
8   *Aspergillius fumigates*. Each organism, except *A.*  
9   *fumigatus*, was grown overnight at 35-37°C in  
10   tryptose broth. *A. fumigates* was grown for 48  
11   hours. Each organism was adjusted to approximately  
12   10<sup>5</sup> organisms/ml with sterile saline. For the  
13   broth dilution test, terpenes were diluted in  
14   sterile tryptose broth to give the following  
15   dilutions: 1:500, 1:1000, 1:2000, 1:4000, 1:8000,  
16   1:16,000, 1:32,000, 1:64,000 and 1:128,000.

17  
18   Each dilution was added to sterile tubes in 5 ml  
19   amounts. Three replicates of each series of  
20   dilutions were used for each test organism. 0.5 ml  
21   of the test organism was added to each series and  
22   incubated at 35-37°C for 18-24 hours. After  
23   incubation the tubes were observed for growth and  
24   plated onto blood agar. The tubes were incubated  
25   an additional 24 hours and observed again. The *A.*  
26   *fumigates* test series was incubated for 72 hours.  
27   The minimum inhibitory concentration for each test  
28   organism was determined as the highest dilution  
29   that completely inhibits the organism.

30

1 Table 2: Results of the inhibitory activity of  
2 different dilutions

Organism	Visual assessment of growth *			Growth after subculture to agar plates *			Mean inhibitory dilution
	1	2	3	1	2	3	
<i>S. typhimurium</i>	500	500	500	500	500	500	500
<i>E. coli</i>	1000	1000	1000	1000	1000	1000	1000
<i>P. mirabilis</i>	1000	1000	1000	1000	1000	1000	1000
<i>S. aureus</i>	1000	1000	1000	1000	1000	1000	1000
<i>C. albicans</i>	1000	1000	1000	1000	1000	1000	1000
<i>A. fumigatus</i>	8000	16000	16000	8000	16000	16000	13300
* The results of the triplicate tests with each organism as the reciprocal of the dilution that showed inhibition/killing							
** NI = not inhibited							

3

4

5 EXAMPLE 6: In in-vitro effectiveness of terpenes  
6 against *Escherichia coli* over time.

7

8 This example demonstrates the effectiveness of  
9 terpenes at several concentrations against  
10 *Escherichia coli* and cultured over time. Terpene  
11 dilutions (1:500, 1:1000, 1:2000, 1:4000, 1:8000,  
12 and 1:16,000) were prepared in BHI broth and in  
13 saline. These were prepared in 25 ml amounts. *E.*  
14 *coli* was grown overnight in BHI broth and diluted  
15 to a MacFarland 0.5 concentration in saline. This  
16 solution was diluted 1:100 to be used to inoculate

1 (0.5 ml) each terpene dilution tube. The series  
 2 that contained the terpene dilution in BHI was  
 3 tested at 30 min, 90 min, 150 min and 450 min.  
 4 Each tube was mixed and serially diluted in saline.  
 5 0.5 ml of each dilution was spread plated onto  
 6 MacConkey (MAC) agar plates. Also, 3 drops of the  
 7 undiluted and the 1:100 dilution was added into  
 8 respective tubes of BHI broth. The tubes and  
 9 plates were incubated overnight at 35°C. The  
 10 series that contained the terpene's dilution in  
 11 saline were tested at 60 min, 120 min, 180 min and  
 12 480 min. Each tube was mixed and serially diluted  
 13 in saline. 0.5 ml of each dilution was spread  
 14 plated onto MacConkey (MAC) agar plates. Also, 3  
 15 drops of the undiluted and the 1:100 dilution were  
 16 added into respective tubes of BHI broth. The  
 17 tubes and plates were incubated overnight at 35°C.  
 18

19 Table 3: Subculture from the tubes containing  
 20 various dilutions of terpenes in broth

Time	Dilution	1:500	1:1000	1:2000	1:4000	1:8000	1:16,000
30 min	Undiluted	NG	+	+	+	+	+
	1:100	NG	+	+	+	+	+
90 min	Undiluted	NG	NG	+	+	+	+
	1:100	NG	NG	NG	+	+	+
150 min	Undiluted	NG	NG	+	+	+	+
	1:100	NG	NG	NG	+	+	+
450 min	Undiluted	NG	NG	+	+	+	+
	1:100	NG	NG	+	+	+	+

21 NG: no growth, +: growth

1 Table 4: Subculture from the tubes containing  
 2 various dilutions of terpenes in saline  
 3

Time	Dilution	1:500	1:1000	1:2000	1:4000	1:8000	Control
60 min	Undiluted	NG	+	+	+	+	+
	1:100	NG	NG	NG	+	+	+
120 min	Undiluted	NG	NG	NG	+	+	+
	1:100	NG	NG	NG	NG	+	+
180 min	Undiluted	NG	NG	NG	+	+	+
	1:100	NG	NG	NG	NG	+	+
480 min	Undiluted	NG	NG	NG	NG	+	+
	1:100	NG	NG	NG	NG	NG	+

4 NG: no growth, +: growth

5

6 Table 5: The quantitative results of the activity  
 7 of various terpene dilutions against *E.coli*  
 8 (cfu)

Media	Time	1:500	1:1000	1:2000	1:4000	1:8000	Control
Broth	30 min	0	0	660	3600	3600	4600
	90 min	0	0	12	4600	5400	7600
	150 min	0	0	10	8000	12,000	14,000
	450 min	0	0	15,000	$28 \times 10^3$	$23 \times 10^7$	$16 \times 10^8$
Saline	60 min	0	4	140	4000	2000	1300
	120 min	0	0	0	90	3800	2600
	180 min	0	0	0	2	2000	5000
	480 min	0	0	0	0	104	8000

9 NG: no growth, +: growth

10

11

12

1 EXAMPLE 7: In vitro effectiveness of selected  
2 terpenes on *Helicobacter pylori*.

3  
4 This example shows the bactericidal effect of  
5 selected terpenes on the viability of *H. pylori*.  
6 Five terpenes (anethole, carvone, citral, geraniol  
7 and b-ionone) were used for this study. Terpenes  
8 were mixed to a ratio of 90% terpene plus 10%  
9 polysorbate-80. The *H. pylori*, used was strain  
10 #26695 of porcine origin, this bacteria is a  
11 motile, cag A, vac A cytotoxin-positive gram  
12 negative bacteria which colonizes gnotobiotic  
13 piglets and indefinitely persists within the  
14 gastric microenvironment as a superficial infection  
15 of the gastric mucosa and mucus layer.

16  
17 The study was as follows:

18  
19 1) Stock solutions of each terpene with  
20 polysorbate-80 were prepared (1.8 ml terpene plus  
21 0.2 ml polysorbate-80).

22  
23 2) Stock solutions were diluted in Brucella broth  
24 10% (v/v) fetal calf serum to a final concentration  
25 of stock at 1:10, 1:50, 1:100, 1:500, 1:1000,  
26 1:5000 and 1:10000. Controls consisted of 10%  
27 (v/v) polysorbate-80 in Brucella broth, Brucella  
28 broth alone and bacteria in Brucella broth.

29  
30 3) A total of  $1.0 \times 10^6$  bacteria (30  $\mu$ l) was added  
31 to 970  $\mu$ l terpene dilutions (final volume of 1.0

1 ml) in loosely capped tubes and incubated for 24  
 2 hours at 37 °C with continuous mixing.  
 3  
 4 4) Duplicate samples (0.1 ml) from each test  
 5 dilution was titrated onto blood agar plates and  
 6 incubated for 48 hours at 37°C on 10% CO<sub>2</sub>  
 7 environment. Bacterial colony forming units (cfu)  
 8 were determined by visual (counting) inspection.  
 9 Recovered bacteria were confirmed to be *H. pylori*  
 10 by catalase and urease enzyme activities.  
 11  
 12 The results are summarized in the following table:  
 13  
 14 Table 6: Effect of different terpenes on *H. pylori*  
 15 growth

	Final dilution tested for antimicrobial effects against 10 <sup>6</sup> cfu							
Terpene	1:10	1:50	1:100	1:500	1:1000	1:5000	1:10000	1:50000
Polysorbate -80	NG*	NG	NG	10 <sup>3</sup>	10 <sup>4</sup>	TNTC**	TNTC	TNTC
Anethole	NG	NG	NG	NG	10 <sup>3</sup>	10 <sup>3</sup>	TNTC	TNTC
Carvone	NG	NG	NG	NG	NG	10 <sup>4</sup>	TNTC	TNTC
Geraniol	NG	NG	NG	NG	NG	NG	10 <sup>2</sup>	TNTC
Citral	NG	NG	NG	NG	NG	NG	NG	TNTC
b-ionone	NG	NG	NG	NG	NG	NG	NG	TNTC

16 \* NG = no growth      \*\* TNTC = Too Numerous To Count

17

18 EXAMPLE 8: In vitro effectiveness of single or  
 19 combination of terpenes against *E. coli*.

20

21 The objective of this example was to determine an  
 22 optimum terpene mixture which could have a greater

1 biocidal effect. *E. coli* strain AW574 was grown in  
2 tryptone broth to an exponential growth phase (O.D.  
3 between 0.4 and 1.0 at 590 nm). One tenth of this  
4 growth was inoculated to 10 ml of tryptone broth  
5 followed by the addition of individual terpenes as  
6 indicated in Example 5; then incubated for 24 hours  
7 at 35-37°C and the O.D. determined in each tube.  
8 The concentration of terpenes was 1 or 2  $\mu$ Mol.  
9 Each treatment was repeated in triplicate. The  
10 results are expressed as percentage bacterial  
11 growth as compared to the control treatment. It is  
12 observed that the combination of terpenes give  
13 better biocidal effect than single terpenes, with  
14 geraniol and carvone better than b-ionone.

15

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31

1 Table 7: Effect of single terpene or their  
2 combination against on *E. coli* growth  
3

$\mu$ Mol terpenes			% Growth
B-ionone	Carvone	Geraniol	
0	0	0	100.00
2	0	0	84.00
0	2	0	63.00
0	0	2	54.00
1	1	1	41.00
1	2	1	31.10
1	1	2	14.80
1	2	2	15.90
2	1	1	48.60
2	2	1	44.30
2	1	2	30.20
2	2	2	1.50

4  
5 EXAMPLE 9: In vitro effectiveness of a combination  
6 of terpenes against different *E. Coli* strains

7  
8 Both well-test and broth test methods were used to  
9 assess the effect of terpene formulations against a  
10 variety of strains of *E. coli*. The broth test  
11 method was judged to be a more applicable  
12 simulation of gastrointestinal tract conditions  
13 than the well plate (zone of inhibition) method. A  
14 series of broth tests was conducted on a selected  
15 test formulation to determine its activity in an  
16 aqueous environment.

17

1   **Test micro-organisms**

2

3   Bacteria were sub-cultured from original American  
4   Type Culture Collection (ATCC) freeze-dried  
5   material. They included *E. coli* strains 8739,  
6   25922 and 700728 (Serotype group O: 157 H:7), which  
7   are BioSafety class 1 organisms and *E. coli* 12795  
8   (Serotype group O: 26) which is a BioSafety class 2  
9   organism. All the bacteria were cultured on  
10   Tryptone Soya Agar (TSA), supplied by Oxoid Ltd,  
11   Hampshire and Mueller Hinton Agar (MHA), supplied  
12   by Merck Ltd. The incubation temperature was  
13   35 °C.

14

15   **Broth Test Procedure**

16

17   *E. coli* cultures were prepared in nutrient broth  
18   and allowed to grow until exponential growth phase  
19   was achieved (16 hours at 35 °C). 1 ml of this  
20   culture was transferred to each of a series of  
21   pre-sterilised Duran bottles containing 100 ml  
22   nutrient broth, 0.5 % w/v Polysorbate 80 and this  
23   gave an initial inoculum of approximately  $10^8$   
24   microbial cells per ml of broth.

25

26   The Duran bottles were agitated on a vortex shaker  
27   to produce good mixing and the Optical Density (OD)  
28   at 590 nm read on a calibrated Unicam UV 300  
29   spectrophotometer controlled by Vision 32 software.  
30   The OD of a sample of a placebo broth was also  
31   recorded.

1 The bottles were then placed in an incubator at  
2 35°C. The bottles were removed at 30 minute  
3 intervals and placed on a vortex shaker at level  
4 three for 30 seconds. The bottles were then  
5 returned to the incubator. The OD was recorded at  
6 hourly intervals, for up to 24 hours.

7  
8 After completion of the tests, the broths were  
9 autoclaved on programme 4 of an AVX240 autoclave  
10 (132 °C for 30 minutes) to sterilise them.

11  
12 The terpenoids tested in this series of exemplary  
13 experiments included I-carvone, citral and geraniol  
14 in varying proportions. One exemplary formulation,  
15 constituting the test formulation, is given in  
16 Table 8, below  
17

Table 8	% w/w
I-Carvone	40
Citral	40
Geraniol	15
Polysorbate 80	5
Total	100

18  
19 This exemplary test formulation was highly active  
20 and clear inhibition of *E. coli* growth was observed  
21 in broth tests conducted at 50 µl and 100 µl doses  
22 in 100 ml broth.

23  
24  
25

1 Table 9: *E. coli* 8739 Broth Test of Formulation  
2 (Optical Density at 590 nm).

3

Time Post Inoculation	Volume of Test Formulation (µl)					
	500	100	50	10	5	Control
0	0.958	0.063	0.019	0.007	0.007	0.003
1	0.708	0.025	0.028	0.024	0.021	0.022
2	0.762	0.023	0.053	0.081	0.087	0.102
3	0.547	0	0.094	0.158	0.179	0.191
4	0.486	0	0.140	0.270	0.286	0.323
5	0.594	0	0.181	0.316	0.311	0.345
6	0.522	0.011	0.238	0.361	0.367	0.401
6.5	0.579	0.014	0.262	0.376	0.372	0.411
23	0.617	0.031	0.285	0.619	0.747	0.654
24	0.553	0.058	0.286	0.606	0.740	0.683

4

5 The 16 hour old *E. coli* culture used as the  
6 inoculum had an OD at 590 nm of 0.697 units.

7

8 Further broth tests were conducted against two  
9 pathogenic strains of *E. coli* (700728, 12795) and  
10 an antimicrobial agent test strain at 50 µl and 100  
11 µl.

12

13

14

15

Table 10: ODs at 16 hours of *E. coli* Cultures used as the Inoculum for the Multi-Strain Broth Test

<i>E.coli</i> test strain	Optical density at 590 nm (absorbance units)
25922	0.852
700728	0.423
12795	0.395

1

2

3

Table 11: Results of a multi-strain Trial of Test formulation

Time Post Inoculation (hours)	<i>E. coli</i> strain/Volume of Test formulation (μl)								
	25922			700728			12795		
	0	50	100	0	50	100	0	50	100
0	0.006	0.040	0.088	0.006	0.032	0.077	0.005	0.040	0.074
1	0.034	0.021	0.031	0.020	0.030	0.028	0.017	0.024	0.035
2	0.129	0.039	0.017	0.075	0.055	0.023	0.055	0.046	0.026
3	0.263	0.074	0.018	0.197	0.106	0.013	0.133	0.101	0.024
4	0.365	0.0138	0.011	0.335	0.188	0.019	0.311	0.198	0.044
5	0.366	0.174	0.004	0.312	0.240	0.050	0.364	0.205	0.049
6	0.395	0.234	0.009	0.371	0.264	0.044	0.399	0.264	0.038
7	0.427	0.256	0.009	0.406	0.287	0.040	0.436	0.282	0.042
23.5	0.688	0.351	0.049	0.545	0.323	0.096	0.564	0.296	0.079
24.5	0.683	0.349	0.054	0.561	0.323	0.106	0.582	0.279	0.083

4

1 Table 9 summarises the results of the 50 and 100  
2 µl/100 ml broth test. These results indicated good  
3 activity against *E. coli* 8739. Table 11 indicates  
4 that the test formulation showed good activity when  
5 challenged with other strains of *E. coli* including  
6 two pathogenic strains 700728, 12795.

7

8 The 100 µl dose of the test formulation had the  
9 lowest OD readings, therefore indicating greater  
10 inhibition of cell proliferation. 50 µl/100 ml  
11 broth of the test formulation appeared to have both  
12 slowed cell proliferation and reduced the final  
13 number of cells present in the broth. Where no  
14 test formulation was present, growth was rapid for  
15 all strains tested, especially in the first 4 hours  
16 after inoculation.

17

18 The test formulation is only one of a range of  
19 terpene formulations investigated so far and it is  
20 clearly very active. Clear inhibition of *E. coli*  
21 growth has been observed in broth tests conducted  
22 at 50 µl and 100 µl/100 ml broth, both against anti  
23 microbial assay strains and against pathogenic  
24 strains.

25

26 Formulations have been developed now which show  
27 very great activity against potentially lethal  
28 strain 0157: H7 of *E. coli*, both at very high  
29 inocula which are not sustainable in life and at  
30 levels which, though likely to be fatal, are found.  
31 Three 100 ml bottles were each filled with McConkey

1 broth to which was added one of either 20 µg/ml  
2 oxacillin, or 10 µg/ml of amoxicillin, or 1 µg/ml  
3 of the exemplary test terpene formulation. Each  
4 bottle was then inoculated with  $10^4$  *E. coli*  
5 0157:H:7 and incubated for 24 hours at 35°C.  
6 Following incubation, the McConkey broth containing  
7 the oxacillin had lost its magenta colour and  
8 become yellowish and turbid, indicating that the  
9 antibiotic had been overwhelmed by the *E. coli*. The  
10 McConkey broth in the bottle containing the  
11 amoxicillin had only slightly reduced magenta  
12 colour, indicating that the antibiotic had  
13 contained the *E. coli*, whereas the McConkey broth  
14 in the bottle containing the terpene sample had an  
15 undiminished magenta colour.

16  
17 This experiment was then repeated under the same  
18 conditions, except that the inoculum of *E. coli*  
19 0157:H:7 was  $10^8$ . In this case, both the oxacillin  
20 and amoxicillin samples were overwhelmed but the  
21 McConkey broth in the bottle containing the terpene  
22 sample had an undiminished magenta colour,  
23 indicating that, even with this extremely high  
24 inoculum, no growth had occurred.

25  
26 Experiments have been carried out on xanthomonads  
27 including assay strains such as *Xylefa multifolia*  
28 and plant pathogens such as *X. fastidiosa*. The  
29 latter causes Pierce's disease which has devastated  
30 grape culture in Southern California and threatens  
31 the wine growing areas of Napa Valley and Sonoma

1 Valley. The organisms are highly susceptible to  
2 terpene formulations according to the present  
3 invention.

4

5 It will be apparent for those skilled in the art  
6 that a number of modifications and variations may  
7 be made without departing from the scope of the  
8 present invention as set forth in the appending  
9 claims.

10

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9

1     **CLAIMS**

2

3     1. An antimicrobial composition for preventing or  
4       treating digestive tract infections, said  
5       composition comprising a terpene or a mixture of  
6       two or more terpenes.

7

8     2. The composition as claimed in Claim 1 which  
9       comprises a mixture of the terpenes carvone and  
10      geraniol.

11

12    3. The composition as claimed in either one of  
13      Claims 1 and 2 which further comprises a  
14      surfactant.

15

16    4. The composition of Claim 3 which consists of 1 to  
17      99% terpenes and 1 to 99% surfactant.

18

19    5. The composition as claimed in either one of  
20      Claims 3 and 4 wherein the terpene or terpene  
21      mixture are natural or synthetic terpenes  
22      selected from citral, b-ionone, geraniol,  
23      carvacrol, eugenol, carvone, terpeniol, anethole  
24      or other generally recognized as safe terpenes  
25      with biocidal properties, and the surfactant is  
26      selected from polysorbate-80, polysorbate-20,  
27      polysorbate-40, polysorbate-60, polyglyceryl  
28      esters, polyglyceryl monooleate, decaglyceryl  
29      monocaprylate, propylene glycol dicaprilate,  
30      triglycerol monostearate or their combination.

31

32

- 1     6. The composition as claimed in any one of Claims  
2         1 to 5 wherein the terpene or terpene mixture is  
3         at least partially encapsulated in a liposome to  
4         form a liposome-terpene(s) combination.  
5
- 6     7. The composition as claimed in Claims 1 to 6  
7         wherein the terpene, terpene mixture or the  
8         liposome-terpene(s) combination is part of the  
9         inner core of a gelatin or cellulose capsule.  
10
- 11    8. The composition as claimed in Claims 1 to 6  
12         wherein the terpene, terpene mixture or the  
13         liposome-terpene(s) combination is freeze dried,  
14         spray dried or dried in order to form a powder  
15         for encapsulation or solubilization.  
16
- 17    9. The composition as claimed in Claims 1 to 6  
18         wherein the terpene, terpene mixture or the  
19         liposome-terpene(s) combination is freeze dried,  
20         spray dried or dried in order to be compressed  
21         in pill or tablet form.  
22
- 23    10. The composition as claimed in Claims 1 to 6  
24         wherein the terpene, terpene mixture or the  
25         liposome-terpene(s) combination is freeze dried,  
26         spray dried or dried in order to be compressed  
27         in pill/tablet and coated for absorption in  
28         different areas along the gastro-intestinal  
29         tract.  
30
- 31    11. A method to prevent or treat microbial  
32         infections of the digestive tract, said method

- 1 comprising orally administering a composition as  
2 claimed in any one of Claims 1 to 10 to patient.
- 3 12. The method as claimed in Claim 11 wherein the  
4 digestive tract infections are produced by  
5 normal, pathogenic or opportunistic  
6 microorganisms or its toxins selected from  
7 *Aerobacter sp.*, *Aspergillus sp.*, *Bacillus sp.*,  
8 *Campylobacter sp.*, *Candida sp.*, *Clostridia sp.*,  
9 *Enterobacteriaceae sp.*, *Enterococcus sp.*,  
10 *Escherichia sp.*, *Haemophilus sp.*, *Helicobacter*  
11 *sp* *Klebsiella sp.*, *Lactobacillus sp.*, *Listeria*  
12 *sp.*, *Propionibacter sp.*, *Pasteurella sp.*,  
13 *Proteus sp.*, *Pseudomonas sp.*, *Salmonella sp.*,  
14 *Shigella sp.*, *Staphylococcus sp.*, *Streptococcus*  
15 *sp.* and *Yersennia sp.*
- 16
- 17 13. The method as claimed in Claim 11 wherein the  
18 terpene, terpene mixture or the  
19 liposome-terpene(s) combination is effective  
20 against pathogenic and normal microflora  
21 comprising of *Aerobacter sp.*, *Aspergillus sp.*,  
22 *Bacillus sp.*, *Campylobacter sp.*, *Candida sp.*,  
23 *Clostridia sp.*, *Enterobacteriaceae sp.*,  
24 *Enterococcus sp.*, *Escherichia sp.*, *Haemophilus*  
25 *sp.*, *Helicobacter sp* *Klebsiella sp.*,  
26 *Lactobacillus sp.*, *Listeria sp.*, *Propionibacter*  
27 *sp.*, *Pasteurella sp.*, *Proteus sp.*, *Pseudomonas*  
28 *sp.*, *Salmonella sp.*, *Shigella sp.*,  
29 *Staphylococcus sp.*, *Streptococcus sp.* and  
30 *Yersennia sp.*
- 31

- 1     14. The method as claimed in Claim 11 wherein the  
2         terpene, terpene mixture or the  
3         liposome-terpene(s) combination is effective  
4         against pathogenic and opportunistic  
5         microorganisms causing traveler's diarrhea.  
6
- 7     15. The method as claimed in Claim 11 wherein the  
8         terpene, terpene mixture or the  
9         liposome-terpene(s) combination is effective  
10        against pathogenic and opportunistic  
11        microorganisms causing ulcers along the  
12        digestive tract.  
13
- 14    16. The method as claimed in Claim 11 wherein the  
15        terpene, terpene mixture or the  
16        liposome-terpene(s) combination is effective  
17        against anthrax.  
18
- 19    17. The method as claimed in Claim 11 wherein the  
20        terpene, terpene mixture or the  
21        liposome-terpene(s) combination is effective  
22        against pathogenic and opportunistic  
23        microorganisms causing scours in calves.  
24
- 25    18. The method as claimed in Claim 11 wherein the  
26        terpene, terpene mixture or the  
27        liposome-terpene(s) combination is effective  
28        against pathogenic and opportunistic  
29        microorganisms causing scours in neonates and  
30        weaned piglets.  
31

- 1     19. The method as claimed in Claim 11 wherein the  
2         terpene, terpene mixture or the  
3         liposome-terpene(s) combination at lower  
4         concentrations has a bacteriostatic effect  
5         against pathogenic and normal gastro-intestinal  
6         microflora.  
7
- 8     20. The method as claimed in Claim 11 wherein the  
9         terpene, terpene mixture or the  
10        liposome-terpene(s) combination at higher  
11        concentrations has a bactericidal effect against  
12        pathogenic and normal gastro-intestinal  
13        microflora.  
14
- 15    21. The method as claimed in any one of Claims 11 to  
16        20 wherein the effective dose of the terpene,  
17        the mixture of terpenes or the  
18        liposome-terpene(s) combination is between 20 mg  
19        and 5000 mg.  
20
- 21    22. The method as claimed in any one of Claims 11 to  
22        20 wherein the effective dose of the terpene,  
23        the mixture of terpenes or the  
24        liposome-terpene(s) combination is between 20  
25        ppm and 50000 ppm in water and/or food consumed  
26        by the human or animal.  
27
- 28    23. The method as claimed in any one of Claims 11 to  
29        22 wherein the terpene, the mixture of terpenes  
30        or the liposome-terpene(s) combination is  
31        prepackaged in liquid form for oral consumption  
32        by humans or animals.

- 1     24. The method as claimed in any one of Claims 11 to  
2         23 wherein the terpene, a mixture of terpenes or  
3         the liposome-terpene(s) combination is mixed  
4         with milk replacer and fed to calves and  
5         piglets.  
6
- 7     25. The method as claimed in any one of Claims 11 to  
8         24 wherein the terpene, the mixture of terpenes  
9         or the liposome-terpene(s) combination is  
10        intubated directly into the stomach of an  
11        animal.  
12  
13

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 02/00015

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/11 A61K31/045 //(A61K31/11,31:045)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, CHEM ABS Data, EPO-Internal, PAJ, MEDLINE, WPI Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOYANOVA LUDMILA ET AL: "Inhibitory effect of rose oil products on Helicobacter pylori growth in vitro: Preliminary report." JOURNAL OF MEDICAL MICROBIOLOGY, vol. 48, no. 7, July 1999 (1999-07), pages 705-706, XP002196283 ISSN: 0022-2615 the whole document	1-5
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 April 2002

Date of mailing of the international search report

03/05/2002

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# INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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